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PRINCIPAL INVESTIGATOR: **Michael B. Jarstfer, PhD**

CONTRACTING ORGANIZATION: **University of North Carolina at Chapel Hill**
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Dr. Michael B. Jarstfer
Co-PIs: Dr. Cort Pedersen, Dr. Sheryl S. Moy

E-Mail: jarstfer@unc.edu

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Please note that this annual report is for a project with three principal investigators: Michael Jarstfer, Cort Pedersen, and Sheryl Moy. .

14. ABSTRACT
Currently, there are no established pharmaceutical strategies that effectively treat core autism spectrum disorder (ASD) symptoms, including pervasive social deficits and repetitive behaviors. The oxytocin pathway has an important role in normal human social behaviors, and oxytocin dysregulation has been implicated in ASD-associated behavioral symptoms. There is now emerging evidence that oxytocin has therapeutic efficacy in ameliorating core ASD symptoms associated with social behavior. However, from the standpoint of drug discovery, oxytocin is a poor candidate as a standard clinical treatment. Oxytocin is rapidly metabolized and has low brain penetrance with peripheral administration. The goal of the proposed studies is to discover new small-molecule compounds that enhance oxytocin signaling, as novel drug interventions for social deficits and abnormal repetitive behavior relevant to ASD. In this second year, we have published our findings that oxytocin can effectively overcome representative ASD phenotypes in two mouse lines that model ASD-like behaviors, including overt alterations in social behavior, and we have extended this work to a genetic model of social impairment, the *Grin1* knockdown mouse. We have recently found that oxytocin has remarkable prosocial effects in *Grin1* mice. We have also evaluated one synthetic oxytocin agonist, Compound 39, and one oxytocin metabolite, for efficacy against social deficits in BALB/cByJ mice. Overall, we have successfully validated three mouse models for as preclinical screens for compounds targeting the oxytocin receptor, and provided leads for a drug discovery campaign for social deficits and other core autism symptoms.

15. SUBJECT TERMS
autism, mouse model, neuropeptides, oxytocin, sociability

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Introduction

Currently, there are no established pharmaceutical strategies that effectively treat core autism spectrum disorder (ASD) symptoms, including pervasive social deficits and repetitive behaviors. The oxytocin pathway has an important role in normal human social behaviors, and oxytocin dysregulation has been implicated in ASD-associated behavioral symptoms. There is now emerging evidence that oxytocin has therapeutic efficacy in ameliorating core ASD symptoms associated with social behavior. However, from the standpoint of drug discovery, oxytocin is a poor candidate as a standard clinical treatment. Oxytocin is rapidly metabolized and has low brain penetrance with peripheral administration. The goal of the proposed studies is to discover new small-molecule compounds that enhance oxytocin signaling, as novel drug interventions for social deficits and abnormal repetitive behavior relevant to ASD. In the first two years of our project, we have established that oxytocin can effectively overcome representative ASD phenotypes in three mouse lines that model ASD-like behaviors, including overt alterations in social behavior and abnormal repetitive behavior. We are currently prioritizing synthetic compounds that activate the oxytocin receptor using cell-based assays, and evaluating the therapeutic efficacy of the top molecules in the characterized mouse lines (presently, Compound 39 and the oxytocin metabolite 4-9). Our research employs a highly-innovative screening paradigm to identify activators of oxytocin function relevant to treatment strategies for ASD. The successful completion of the proposed aims will validate the oxytocin receptor as a small-molecule drug target for the amelioration of ASD-associated phenotypes, contribute to the drug discovery process by validating mouse models for preclinical testing, and provide leads for a drug discovery campaign directed at the oxytocin receptor.

Body

Validation of mouse models as preclinical efficacy screens. We have previously observed low sociability in adolescent mice from the BALB/cByJ (Moy *et al*, 2007) and C58/J (Moy *et al*, 2008; Ryan *et al*, 2010) inbred strains. We are now excited to report our recent publication (Appendix 2) that describes how the lack of social preference in both the BALB/cByJ and C58/J models can be reversed by a 2-week chronic regimen of oxytocin (1.0 mg/kg) treatment (Teng *et al*, 2013). A single dose of oxytocin was ineffective. Our findings are the first evidence that the two inbred strain models can be utilized as preclinical screens for prosocial effects of novel compounds active at the oxytocin receptor. A particularly intriguing aspect of our findings in the BALB/cByJ mice is that prosocial effects of oxytocin can be observed 24 hours after the final oxytocin treatment. We have recently extended these findings to show that social deficits are still rescued 48 hours following oxytocin treatment, demonstrating the persistence of the beneficial effects (**Figure 1**).

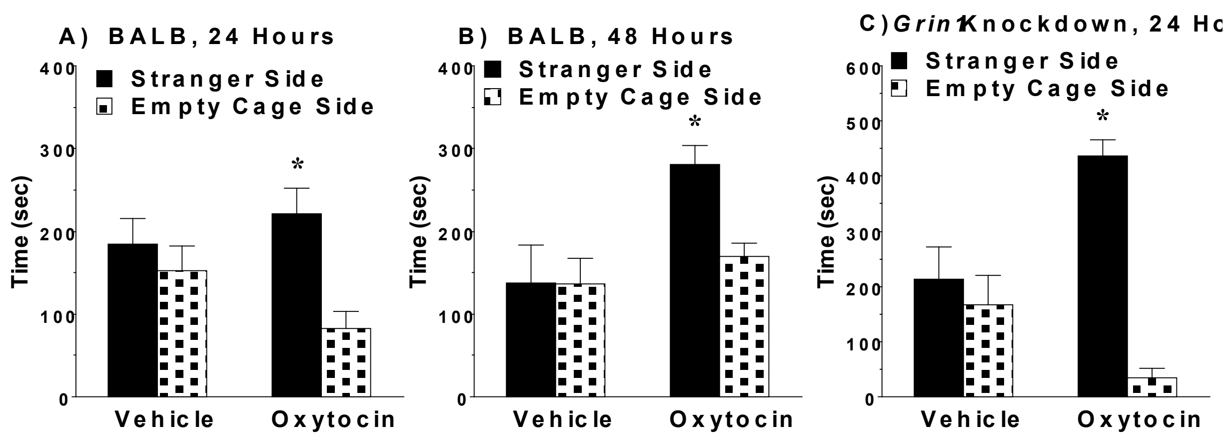

Figure 1. Persistent prosocial effects of chronic oxytocin in BALB/cByJ and *Grin1* knockdown mice. Subjects were given a sub-chronic regimen of oxytocin (4 doses, with at least 48 hours between each dose), and then tested for sociability either 24 hours (A and C) or 48 hours (B) following the final treatment. At each time point, only the oxytocin-treated mice had a preference for spending more time near an unfamiliar stranger mouse, versus an empty wire cage. * p<0.05, within-group comparison.

In this project, we also proposed to evaluate a genetic mouse model of autism-like phenotypes, the *Grin1* knockdown mouse. The *Grin1* gene encodes the NR1 subunit of the NMDA receptor. Mice with reductions in *Grin1* demonstrate significant alterations in social behavior and deficits in social approach, as well as impairments in habituation and aberrant reactivity to sensory stimuli (Duncan et al. 2004; Moy et al. 2012). We have recently determined that a sub-chronic regimen of oxytocin has powerful prosocial efficacy in male *Grin1* mice (**Figure 1C**), and we are currently testing female *Grin1* mice in the same treatment regimen.

Evaluation of oxytocin agonists in the BALB/cByJ mouse model. This past year, we evaluated two doses of Compound 39, a synthetic oxytocin agonist, for prosocial efficacy. The lower dose (30

mg/kg) was selected as one which did not produce any sedative-like effects on locomotor behavior. We chose the higher dose (50 mg/kg) based on oxytocin-like effects in an acoustic startle test. However, sub-chronic treatment regimens with either the higher or lower dose of Compound 39 failed to rescue social deficits in the BALB mice (**Figure 2A**). On the other hand, sub-chronic treatment with the oxytocin (4-9) metabolite led to significant increases in social preference (**Figure 2B**), suggesting that fragments of the oxytocin nonapeptide might have full prosocial potency.

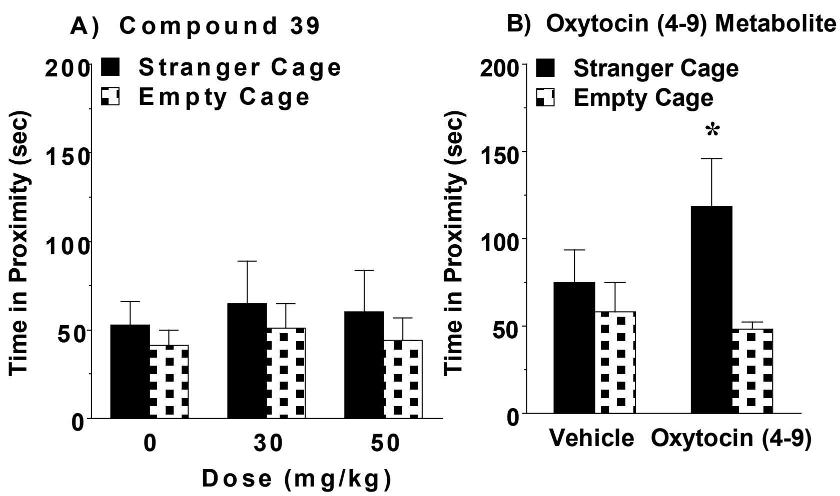

Figure 2. Prosocial effects of oxytocin (4-9) metabolite, but not Compound 39 (a synthetic oxytocin agonist) in BALB/cByJ mice. Subjects were given a sub-chronic treatment regimen (4 doses, with at least 48 hours between each dose), and then tested for sociability 24 hours following the final treatment. Measures were taken of time spent in proximity to an unfamiliar stranger mouse, versus an empty wire cage. *$p<0.05$, within-group comparison.

Prioritization of positive allosteric modulators and agonists. We previously completed a high throughput screen of over 300,000 compounds in order to identify compounds that activate the oxytocin receptor. All initial positive agonists (199 compounds) and allosteric modulators (65 compounds) have been confirmed using fluorescence-based assays. Overall, most agonists were moderately potent (EC_{50} ranged from 5 to 100 μM). However, agonists displayed little selectivity for the oxytocin receptor when compared to vasopressin receptors. These results were validated with Ca^{2+} release assays as well as IP_3 formation assays (data not shown). We have examined several series of analogs based on active hits, but have not achieved increased specificity. To further our efforts towards new oxytocin (OT) receptor agonists, we examined several oxytocin fragments that are expected to be metabolites of oxytocin. We are still in the process of testing these fragments against all human membrane-bound receptors, and to date have shown that they do not have activity against human orphan receptors. As described upon, we also tested OT(4-9) in a sociability task and found it to be active. We therefore have decided to more fully characterize Cmpd39 and OT(4-9), compare with other synthetic oxytocin agonists that are cmpd39 derivatives, and smaller oxytocin fragments in year three of the project.

Allosteric modulators, which are more desirable, were generally less potent with maximum increase in response to oxytocin near 10 percent. In order to optimize potency, we have continued exploring analoging of derivatives in order to optimize their activity. To date, we have screened 55

analogs of two initial hits (a functionalized amino acid and a pyrazolopyrimidine) in the positive allosteric modulator assay. In year two, we assayed the pyrazolopyrimidine analogs (**Figure 3**). The data revealed that even at high concentrations, analogs of these two initial hits are not potent enough to warrant further analysis.

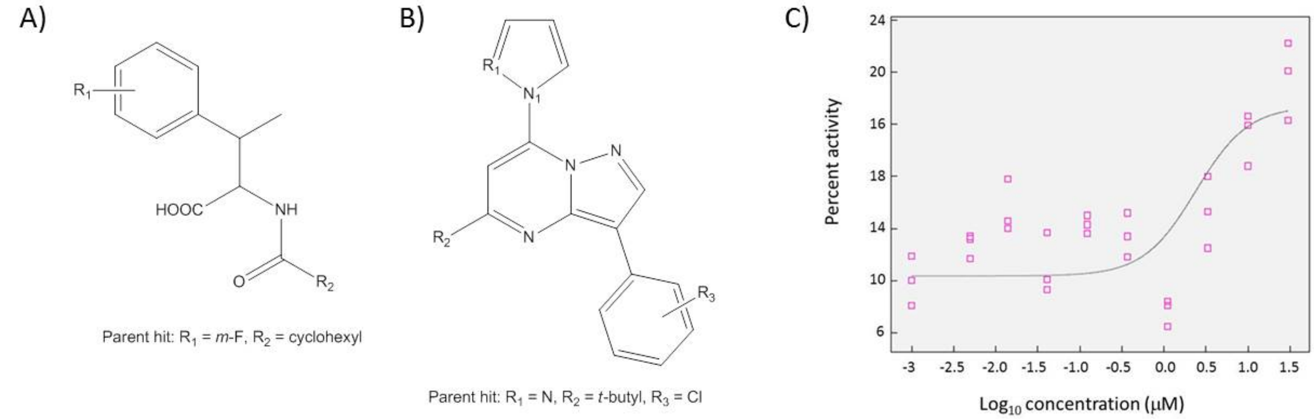

Figure 3. Analysis of positive allosteric modulators of the human oxytocin receptor. Two series of analogs based on initial hits from our high throughput screen for positive allosteric modulation of the human oxytocin receptor have been analyzed. (A) Amino acid derivatives tested in year one. (B) Pyrazolopyrimidine derivatives tested in year two. (C) Example dose response for the most potent pyrazolopyrimidine derivative, the parent compound, in an allosteric modulator assay. In short, CHO cells stably transfected with the oxytocin receptor were treated with test compound and 1 nM oxytocin and the oxytocin receptor response was detected by calcium accumulation. Triplicate data were fit by nonlinear regression.

Plans for prioritization of positive allosteric modulators and agonists. In year two, we examined analogs from several agonist series in an effort to separate vasopressin receptor activity from oxytocin receptor activity and 2 positive allosteric modulator (PAM) series. In total now, we have examined analogs based on eight hits from our initial screen. We have evaluated the tractability of a third PAM. We will obtain ~15 analogs of this positive allosteric modulators and will complete screening in cell based assays during the third year of the award period.

Key Research Accomplishments

1. Demonstrated that sub-chronic oxytocin treatment has highly significant prosocial effects in *Grin1* knockdown mice, a genetic model of glutamatergic hypofunction.

2. Evaluated Compound 39 and the oxytocin (4-9) metabolite in the BALB/cByJ mouse model.

3. Evaluated derivatives of initial positive allosteric modulator hit in cell-based assays.

4. Key accomplishments in relationship to proposed Statement of Work: we have completed subtasks 1a, 1b, 2a, 2b, 2c, 3a, 3b, 3c, 3d (partial), 4a, 4b, and initiated 2d, 5a, 5b, 6e (see appendix for proposed Statement of Work).

Reportable Outcomes

1. The research team, including Dr. Brian Teng, the post-doctoral fellow recruited for this project, have published a manuscript on the validation of two mouse models as screens for oxytocin effects on social deficits and repetitive behavior (**Appendix II**).
2. The results of the our research, including the synthetic oxytocin receptor agonist Compound 39, were presented at the International Meeting for Autism Research May 2-4, 2013. The full abstract is in Appendix II.

Conclusion

In the first 23 months of the funded project, we have made significant progress, particularly on validating our proposed mouse models and in determining the ability of non-peptide synthetic compounds to affect the oxytocin receptor. We demonstrated that oxytocin can reverse social deficits in C58/J, BALB/cByJ and *Grin1* knockdown mice. Most remarkably, we showed that, in each case, chronic oxytocin treatment was required to promote prosocial activity. This is an important observation that will inform both preclinical screening, as well as clinical trials. Surprisingly, prosocial effects emerged one or two weeks following treatment in the C58/J mice. The results with the mouse models indicate that oxytocin treatment results in molecular changes that are more complex and long lasting than simply activating the oxytocin receptor. This is the subject of studies we will conduct as part of subsequent proposals and was the subject of the post-doctoral fellowship awarded to Dr. Brian Teng, who conducted the experiments described here.

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Teng BL, Nonneman RJ, Agster KL, Nikolova VD, Davis TT, Riddick NV*, et al* (2013). Prosocial effects of oxytocin in two mouse models of autism spectrum disorders. *Neuropharmacology* **72**: 187-196.

Appendix I

Statement of Work

NOTE: There is one animal site for all experiments: the Mouse Behavioral Phenotyping Laboratory, the corresponding PI is Sheryl Moy, though each PI will participate in experimental design and data interpretation.

<u>Task 1.</u> Establish the effect of oxytocin on the behavior of *Grin1*$^{neo/neo}$ mice (Major contribution from Drs. Moy and Pedersen, minor contribution from Dr Jarstfer). **Performance site: UNC Chapel Hill, Mouse Behavioral Phenotyping Laboratory, Medical Biomolecular Research Building.**

Number of mice: 183

<u>Subtask 1a</u>. Submit forms for IACUC approval of animal studies (month 1).

<u>Subtask 1b</u>. Submit ACURO Animal Use Appendix for Research Involving Animals documents for review and approval of animal studies (months 1-4).

<u>Subtask 1c</u>. Rear and genotype *Grin1*$^{neo/neo}$ mice and wild type controls (months 4-36).

<u>Subtask 1d.</u> Test the effect of oxytocin on the behavior of *Grin1*$^{neo/neo}$ mice in the social approach test (months 6-12). The social approach test uses an automated apparatus for quantitation of social approach behaviors (as described in Moy et al., *Genes, Brain and Behavior* (2004) 3: 303–314). In short, time spent in each chamber and the number of entries are scored automatically by a system detecting photocell beam breaks. We also measure time sniffing and sniffing bouts.

<u>Task 2.</u> Establish the effect of oxytocin on the behavior of C58/J mice. (Major contribution from Drs. Moy and Pedersen, minor contribution from Dr Jarstfer). **Performance site: UNC Chapel Hill, Mouse Behavioral Phenotyping Laboratory, Medical Biomolecular Research Building.**

Number of mice: 143

<u>Subtask 2a</u>. Submit forms for IACUC approval of animal studies (month 1).

<u>Subtask 2b</u>. Submit ACURO Animal Use Appendix for Research Involving Animals documents for review and approval of animal studies (months 1-4).

<u>Subtask 2c</u>. Test the effect of oxytocin on the behavior of C58/J mice in the social approach test (months 6-12).

Subtask 2d. Test the effect of oxytocin on the behavior of C58/J mice in the repetitive behavior test (months 12-18).

Task 3. Establish the effect of oxytocin on the behavior of BALB/cByJ mice. (Major contribution from Drs. Moy and Pedersen, minor contribution from Dr Jarstfer). **Performance site: UNC Chapel Hill Mouse Behavioral Phenotyping Laboratory, Medical Biomolecular Research Building**

Number of mice: 84

Subtask 3a. Submit forms for IACUC approval of animal studies (month 1).

Subtask 3b. Submit ACURO Animal Use Appendix for Research Involving Animals documents for review and approval of animal studies (months 1-4).

Subtask 3c. Test the effect of oxytocin on the behavior of BALB/cByJ mice in the social approach test (months 6-12).

Subtask 3d. Publish findings from tasks 1-3 in scholarly journal.

Task 4. Validate active compounds from the high throughput screen. (Major contribution from Jarstfer). **Performance sites: UNC Chapel Hill Genetics Medicine Building (FLIPR assays) and Beard Hall (data analysis and interpretation).**

Subtask 4a. Confirm and characterize active agonists from the initial high throughput screen using resynthesized compounds (months 1-4)

Subtask 4b. Confirm and characterize active positive allosteric modulators from the initial high throughput screen using resynthesized compounds (months 2-5)

Task 5. Identify lead compounds for testing in animal models. (Major contribution from Jarstfer).

Performance site: UNC Chapel Hill, Genetic Medicine building.

For Task 5, the Specialized Chemistry Center for Accelerated Probe Development will perform synthesis of compounds based on the activity we observe in our cell-based assays. They will perform a service – both intellectually and physically – by making molecules and determining potential derivatives to optimize activity. No cell-based or animal assays will be conducted at the Center. In Task 4, the compounds are the initial hits from the high throughput screen. In Task 5, the compounds are derivatives of the hits, either synthetic or commercially available compounds.

Subtask 5a. Conduct iterative syntheses and testing of agonists in collaboration with the Vanderbilt Specialized Chemistry Center for Accelerated Probe Development (months 4-24). We will test between 50 and 200 compounds.

Screening will be conducted using first the cell-based calcium release assay we used in the HTS. Positives will be further analyzed using IP3 release assays. Counter assays using untransfected cells and cells expression the vasopressin receptor will confirm selectivity.

Subtask 5b. Conduct iterative syntheses and testing of positive allosteric modulators in collaboration with the Vanderbilt Specialized Chemistry Center for Accelerated Probe Development (months 5-24).

We will test between 50 and 200 compounds.

Screening will be conducted using first the cell-based calcium release assay we used in the HTS. Unlike the agonist assay, these assays will include a low level of oxytocin to prime the receptor. Positives will be further analyzed using IP3 release assays. Counter assays using untransfected cells and cells expression the vasopressin receptor will confirm selectivity.

Subtask 5c. Analysis of safety and untoward activity of potential test compounds (Months 18-28). Compounds will be screened in cell based assays for activity against other receptors. In particular, assays for bioavailability predictions (CaCo2, MDR-1) and cardiovascular toxicity predictions (HERG, 5-HT2B) will be conducted.

Subtask 5d. Publish identification of new oxytocin receptor agonists and positive allosteric modulators.

Task 6. Establish the effect of small molecule agonists on the behavior of ASD-model mice (Major contribution from Drs. Moy and Pedersen, minor contribution from Dr Jarstfer). **Performance site: UNC Chapel Hill Mouse Behavioral Phenotyping Laboratory, Medical Biomolecular Research Building**

Agonists will be tested without the addition of oxytocin. Endogenous oxytocin will be present at endogenous levels, but we will not add addition oxytocin.

Subtask 6a. Determine appropriate dose of test compounds in C57BL/6J mice using acoustic startle test (months 18-24).

Subtask 6b. Test the effect of target compounds on the behavior of *Grin1*$^{neo/neo}$ mice in the social approach test (months 24-36).

Subtask 6c. Test the effect of target compounds on the behavior of C58/J mice in the social approach test (months 24-36).

Subtask 6d. Test the effect of target compounds on the behavior of C58/J mice in the repetitive behavior test (months 24-36).

Subtask 6e. Test the effect of target compounds on the behavior of BALB/cByJ mice in the social approach test (months 24-36).

Appendix II

International Meeting for Autism Research abstract

Mouse Models of Autism Phenotypes As Preclinical Screening Platforms for Novel Oxytocinergic Compounds

B. L. Teng1,2, R. J. Nonneman1,3, V. D. Nikolova1,4, K. L. Agster1,4, T. T. Davis1,2, N. V. Riddick1,4, L. K. Baker1, C. A. Pedersen1,4, M. B. Jarstfer1,2 and S. S. Moy1,4,

(1)Carolina Institute for Developmental Disabilities, University of North Carolina School of Medicine, Chapel Hill, NC, (2)UNC Eshelman School of Pharmacy, University of North Carolina, Chapel Hill, NC, (3)Department of Genetics, University of North Carolina School of Medicine, Chapel Hill, NC, (4)Department of Psychiatry, University of North Carolina School of Medicine, Chapel Hill, NC

Background: There is emerging evidence that oxytocin (OT) treatment can improve social deficits and repetitive behavior in autism spectrum disorders (ASDs). However, administration of the neuropeptide, which has a short plasma half-life and poor ability to penetrate the blood-brain barrier, is a problematic issue for clinical use. We have recently initiated a drug development program to identify novel, highly selective, non-peptide OT receptor (OTR) agonists. These efforts would be accelerated by animal models to screen drug candidates for efficacy against ASD-relevant phenotypes.

Objectives: Validate mouse models for preclinical screening of compounds that target the OT pathway, in order to facilitate the development of therapeutics for core ASD symptoms. Methods: BALB/cByJ and C58/J are well-characterized inbred mouse strains that exhibit behavioral phenotypes relevant to ASD. To validate these models as preclinical screens, mice were tested for OT effects on sociability in a three-chamber task and perseverative responses in a marble-burying assay. C58/J was also examined for OT effects on repetitive behavior and open field activity. These screening platforms were then used to evaluate Compound 39 (a synthetic, non-peptide OTR agonist).

Results: The acute OT regimen did not increase sociability in BALB/cByJ. However, the sub-chronic OT regimen (i.e. four intraperitoneal injections across 7-8 days) had significant prosocial effects in both BALB/cByJ and C58/J. Increased sociability was observed 24 hr following the final OT dose in BALB/cByJ, while prosocial effects of OT emerged 1-2 weeks post-treatment in C58/J. An acute OT regimen decreased motor stereotypy in C58/J, at a dose that did not produce sedative or anxiolytic-like effects in open field testing. Similarly, acute OT treatment led to significant reductions in marble-burying by BALB/cByJ. Consistent with previous research, Compound 39 produced some OT-like effects; however, the drug had no effect on sociability.

Conclusions: These studies show that OT reverses social deficits in mouse models of ASD, dependent on dose regimen and genotype. Furthermore, acute OT decreases abnormal repetitive behavior in C58/J and marble-burying in BALB/cByJ. These findings provide validation of the BALB/cByJ and C58/J models as valuable platforms for screening novel drugs for intervention in ASDs, and for elucidating the mechanisms contributing to prosocial and other beneficial effects of OT.

Publication from work.

Teng, B.L., Nonneman, R.J., Agster, K.L., Nikolova, V.D., Davis, T.T., Riddick, N.V., Baker, L.K., Pedersen, C.A., Jarstfer, M.B., Moy, S.S. (2013) Prosocial effects of oxytocin in two mouse models of autism spectrum disorders. *Neuropharmacology* **72**: 187-196.

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Prosocial effects of oxytocin in two mouse models of autism spectrum disorders

Brian L. Teng [a,d,*], Randal J. Nonneman [a,c], Kara L. Agster [a,b], Viktoriya D. Nikolova [a,b], Tamara T. Davis [a,d], Natallia V. Riddick [a,b], Lorinda K. Baker [a], Cort A. Pedersen [a,b], Michael B. Jarstfer [a,d], Sheryl S. Moy [a,b]

[a] *Carolina Institute for Developmental Disabilities, University of North Carolina School of Medicine, Chapel Hill, NC 27599, USA*
[b] *Department of Psychiatry, University of North Carolina School of Medicine, Chapel Hill, NC 27599, USA*
[c] *Department of Genetics, University of North Carolina School of Medicine, Chapel Hill, NC 27599, USA*
[d] *UNC Eshelman School of Pharmacy, University of North Carolina, Chapel Hill, NC 27599, USA*

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ABSTRACT

Clinical evidence suggests that oxytocin treatment improves social deficits and repetitive behavior in autism spectrum disorders (ASDs). However, the neuropeptide has a short plasma half-life and poor ability to penetrate the blood–brain barrier. In order to facilitate the development of more bioavailable oxytocinergic compounds as therapeutics to treat core ASD symptoms, small animal models must be validated for preclinical screens. This study examined the preclinical utility of two inbred mouse strains, BALB/cByJ and C58/J, that exhibit phenotypes relevant to core ASD symptoms. Mice from both strains were intraperitoneally administered oxytocin, using either acute or sub-chronic regimens. Acute oxytocin did not increase sociability in BALB/cByJ; however, sub-chronic oxytocin had significant prosocial effects in both BALB/cByJ and C58/J. Increased sociability was observed 24 h following the final oxytocin dose in BALB/cByJ, while prosocial effects of oxytocin emerged 1–2 weeks post-treatment in C58/J. Furthermore, acute oxytocin decreased motor stereotypy in C58/J and did not induce hypoactivity or anxiolytic-like effects in an open field test. This study demonstrates that oxytocin administration can attenuate social deficits and repetitive behavior in mouse models of ASD, dependent on dose regimen and genotype. These findings provide validation of the BALB/cByJ and C58/J models as useful platforms for screening novel drugs for intervention in ASDs and for elucidating the mechanisms contributing to the prosocial effects of oxytocin.

1. Introduction

Autism spectrum disorders (ASDs), which occur in approximately 1% of the population, are characterized by core deficits in sociability and communication skills, as well as abnormal restrictive and repetitive behaviors (CDC, 2012; Elsabbagh et al., 2012; Nazeer and Ghaziuddin, 2012). Although clinical evidence suggests that some medications may alleviate repetitive behavior in ASDs (e.g. atypical antipsychotics and selective serotonin reuptake inhibitors), these drugs have not proven to be consistently effective and have been associated with significant adverse side effects (Carrasco et al., 2012; McDougle et al., 2005; McPheeters et al., 2011; Stachnik and Nunn-Thompson, 2007). Furthermore, there are no pharmacological interventions for treating the social deficits associated with ASDs; however, the oxytocin signaling pathway is emerging as a promising avenue for ASD drug discovery efforts (Meyer-Lindenberg et al., 2011; Striepens et al., 2011).

Oxytocin is a neuropeptide hormone with a long recognized role in maternal responses, but there is increasing evidence that oxytocin mediates other aspects of social behavior, and that disruption of normal oxytocin function could lead to impaired sociability and affiliative interactions (Insel, 2010). In line with this premise, several reports indicate oxytocin signaling may be deficient in ASDs (Higashida et al., 2012; Striepens et al., 2011). Thus, pharmacological activation of central oxytocin receptors could have beneficial effects on core ASD symptoms, especially social deficits. This hypothesis is supported by studies that demonstrate acute high doses of oxytocin can improve social function and reduce repetitive behavior in individuals with ASD (Andari et al., 2010;

* Corresponding author. UNC Eshelman School of Pharmacy, Division of Chemical Biology and Medicinal Chemistry, University of North Carolina, 308 Beard Hall, Campus Box 7568, Chapel Hill, NC 27599-7568, USA. Tel.: +1 919 966 8654.
E-mail addresses: brianlteng@unc.edu, brianlteng@gmail.com (B.L. Teng).

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Guastella et al., 2010; Hollander et al., 2007, 2003). However, the clinical utility of oxytocin is limited by its short half-life, poor ability to cross the blood–brain barrier, and affinity for vasopressin receptors (Chini and Manning, 2007; Kang and Park, 2000; Morin et al., 2008; Schorscher-Petcu et al., 2010). These concerns underscore the need to explore the development of selective, non-peptide drugs to target the oxytocin pathway. To achieve this goal, appropriate small animal models are critical for preclinical efficacy testing of novel oxytocinergic compounds.

Previously, we screened multiple commercially-available inbred mouse strains for abnormal phenotypes relevant to core symptoms of human developmental disorders, and identified strains that could serve as appropriate behavioral models for ASDs (Moy et al., 2004, 2008, 2007). For example, we found that specific strains have deficient sociability in a three-chambered choice task, which measures the time a test mouse spends in proximity to a stranger mouse versus an empty cage (i.e. non-social object) (Moy et al., 2008, 2007; Nadler et al., 2004). One of these strains, BALB/cByJ, exhibited both a lack of social preference and high levels of anxiety-like behavior, which could reflect the comorbid anxiety frequently observed in ASDs (Brodkin, 2007). BALB/cJ, a related sub-strain, also has impaired sociability in a three-chambered choice task (Brodkin et al., 2004; Sankoorikal et al., 2006), as well as deficient ultrasonic vocalization during social interaction, which may be relevant to core communication deficits observed in ASDs (Kikusui et al., 2011; Panksepp et al., 2007).

C58/J is another inbred mouse strain that shows low sociability in the three-chambered choice task (Moy et al., 2008; Ryan et al., 2010). C58/J mice also have deficits in social transmission of food preference, a test used to model social communication, and exhibit overt, abnormal repetitive behavior (Ryan et al., 2010). At an early age, C58/J mice spontaneously develop motor stereotypies, which include backflipping, “jackhammer” jumping, and upright scrabbling (Ryan et al., 2010). These robust ASD-like phenotypes in social and repetitive behaviors make the C58/J strain an attractive model for the preclinical evaluation of drug candidates to treat autism. Thus far, one study has identified an agent (GRN-529, a negative allosteric modulator of the metabotropic glutamate receptor subtype 5) that has efficacy in reducing the repetitive behavior of C58/J mice, but the study did not examine drug effects on the lack of sociability in this strain (Silverman et al., 2012b).

Overall, the oxytocin receptor is among the most promising targets for intervention in ASDs, which highlights the need for translational models to assess the behavioral pharmacology of therapeutics targeting the oxytocin pathway (including oxytocin itself). In the present studies, we utilized BALB/cByJ and C58/J to evaluate the effects of oxytocin treatment on behavioral phenotypes relevant to core ASD symptoms. Using both inbred strains allowed the identification of prosocial oxytocin effects that were dependent on genetic background, and the ability to investigate oxytocin efficacy against aberrant repetitive behavior in C58/J.

2. Methods and materials

2.1. Animals

For the BALB/cByJ model, cohorts of male mice (3–4 weeks old, $n = 12$–24) were obtained from Jackson Laboratory (JAX; Bar Harbor, ME). For the C58/J model, mice were offspring of C58/J breeding pairs (JAX) weaned at postnatal day 21 and caged with same-sex littermates. Dams were fed ProLab RMH 2000 and all other mice were fed ProLab RMH 3000 ad libitum with free access to water. Mice were maintained in groups of 2–4 animals per polycarbonate mouse cage lined with Bed-o'Cobs bedding. For enrichment, each cage contained a small section of PVC pipe and two nestlet squares. Mice were housed in an animal facility at The University of North Carolina at Chapel Hill (UNC) in a room with a 12-h light/dark cycle (lights off at 7 pm). All animal care and procedures were conducted in strict compliance with the animal welfare policies set by the National Institutes of Health and UNC, and were approved by the UNC Institutional Animal Care and Use Committee.

2.2. Drug treatment

Oxytocin (Bachem, Torrance, CA) was dissolved in saline containing 0.002% acetic acid. For acute treatments, mice were given a single intraperitoneal (IP) injection of vehicle or 1.0 mg/kg oxytocin 50 min prior to testing for sociability (BALB/cByJ model; Fig. 1), or for repetitive behavior (C58/J model). For sub-chronic treatments (both models), mice were given four IP injections of vehicle or 1.0 mg/kg oxytocin across 8–9 days, with at least 48 h between each injection; behavioral tests

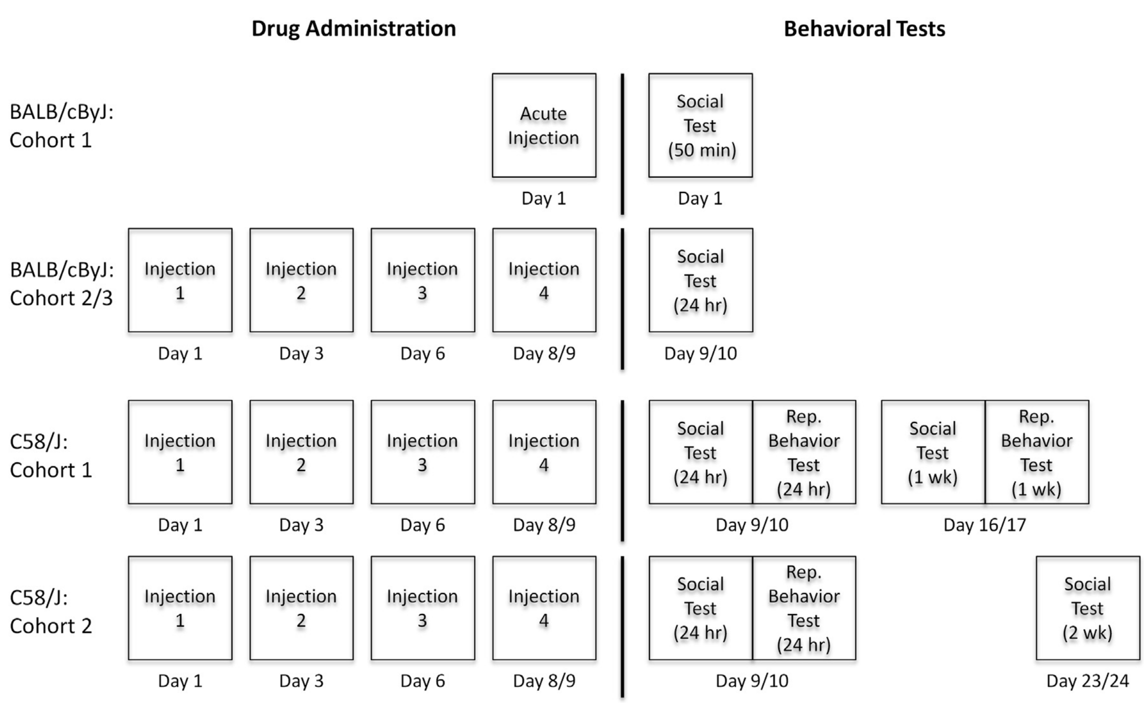

Fig. 1. Experimental timelines for the acute and sub-chronic oxytocin regimens, and behavioral tests in BALB/cByJ and C58/J mice. The acute regimen was a single-dose of vehicle or oxytocin (1.0 mg/kg) administered 50 min before behavioral testing. The sub-chronic regimen consisted of four doses of either vehicle or oxytocin (1.0 mg/kg) across an 8–9 day period, with at least 48 h between each IP injection. Sociability (Social) or repetitive (Rep.) behavior testing occurred on the indicated days. C58/J Cohorts 3 and 4 are not shown.

occurred approximately 24 h after the last injection (Fig. 1). This sub-chronic regimen was designed to reduce the stress that might be found with daily injections, while enhancing sensitivity to oxytocin, as observed with other drugs (Fiorino and Phillips, 1999; Moy and Breese, 2002). Considering the pharmacokinetic properties of oxytocin, many groups have used intracerebroventricular (ICV) injection for acute oxytocin treatments; however, this route induces significant stress and sometimes requires anesthesia in mice, which could interact with oxytocin effects and would not be ideal for repeated administration (Kim et al., 1998; Yamakage et al., 2002). The IP route of administration was selected for rapid drug absorption and relatively minimal stress for the animals. Importantly, other groups have shown that acute IP injection of 1.0 mg/kg oxytocin in mice has antidepressant-like, anti-nociceptive, and autonomic nervous system effects, suggesting that oxytocin is able to enter the central nervous system by this method (Arletti and Bertolini, 1987; Lundeberg et al., 1994; Ring et al., 2006). Experimenters blind to drug identity performed all injections and behavioral tests during daytime (light phase) hours (10 am–4 pm).

2.3. Behavioral tests

2.3.1. BALB/cByJ model

Effects of acute and sub-chronic oxytocin on social preference in the three-chambered choice task were evaluated in separate cohorts of male mice, 6–8 weeks of age at time of testing. The study focused on male mice, in accordance with the higher incidence of autism observed in male versus female children. BALB/cByJ Cohort 1 ($n = 6$ per treatment group; 6–7 weeks of age) was tested for acute oxytocin effects on sociability in the three-chambered choice task (Fig. 1). Two separate BALB/cByJ cohorts were tested following sub-chronic oxytocin treatment: Cohort 2 ($n = 12$ per treatment group; 8–9 weeks of age at time of testing) and Cohort 3 ($n = 15$–18 per treatment group; 7–8 weeks of age at time of testing) were each tested in the three-chambered choice task approximately 24 h following the final dose of vehicle or oxytocin (Fig. 1).

2.3.2. C58/J model

Male and female mice (15–16 of each sex per treatment group; 7–8 weeks of age at time of testing) were used to evaluate the effects of sub-chronic oxytocin treatment on both social preference and repetitive behavior. Both male and female C58/J mice were included in this study, because each displays profound ASD-relevant phenotypes (Ryan et al., 2010), and were available from in-house breeding. For the sociability study, all mice were tested approximately 24 h following the sub-chronic oxytocin regimen. In order to evaluate possible long-term effects of sub-chronic oxytocin exposure, half of the mice were re-tested for sociability 1 week after the final injection (C58/J Cohort 1), and the remaining mice were re-tested 2 weeks after the final injection (C58/J Cohort 2) (Fig. 1). Test groups were balanced as much as possible for treatment and sex. C58/J Cohort 1 (6–8 per treatment group per sex) was further assessed for repetitive behavior 3–4 h following the sociability test at the 24-h and 1-week time points (Fig. 1).

C58/J Cohort 3 (11 males and 10 females; 4–6 months in age) was evaluated for acute oxytocin effects on repetitive behavior using a crossover study design. This crossover design was executed such that half of the mice received vehicle while the other half received oxytocin for the first test, then one week later, the reverse treatments were given for a second test. Thus, each mouse was given two tests, one with vehicle pretreatment and one with oxytocin pretreatment, with one week between the tests. In order to determine the acute effects of oxytocin on general activity, C58/J Cohort 4 (8 males and 8 females; 4–5 months in age) was tested in an open field test, using the same crossover design.

2.4. Three-chambered choice test

Social approach was assessed using an automated three-chambered box (Moy et al., 2007; Nadler et al., 2004) with retractable doorways in each dividing wall to allow entry into each chamber. Entries and time spent in each chamber of the social text box were measured by photocells embedded in each doorway. The choice test had two 10-min phases. The first phase was habituation – the test mouse was first placed in the middle chamber, then the doorways were opened to the side chambers (empty) in order to allow 10 min of free exploration. The second phase was sociability – after the habituation period, the test mouse was enclosed in the center compartment of the social test box, then an unfamiliar stranger (a sex-matched C57BL/6J adult mouse) was placed in a wire cage in one side chamber, while an empty wire cage was placed in the other side. The stranger mouse's location was alternated between the left and right chambers of the social test box across subjects. The doors were then reopened, and the subject was allowed to explore the entire social test box for 10 min. Measures were taken of the time spent in each chamber and number of entries into each chamber by the automated testing system. In addition, a human observer scored time spent sniffing each wire cage, using previously described software (Johns et al., 1998).

2.5. Repetitive behavior in C58/J

To assess repetitive behavior, each subject was placed into a clean home cage and video recorded for 20 min (acute regimen) or 30 min (sub-chronic regimen). The videos were scored for motor stereotypy, grooming, and locomotion using Observer XT (Noldus Information Technology, Leesburg, VA). Two types of motor stereotypy were scored: jackhammer jumping and backflipping, as previously defined (Ryan et al., 2010). These behaviors were selected as the predominant forms exhibited by C58/J (Ryan et al., 2010). Locomotion was coded during bouts of ambulation in which the animal took at least 3 steps (with not more than 1 s between steps). The total numbers of jumps and backflips were combined for the motor stereotypy score, while grooming and locomotion were scored for duration.

2.6. Open field activity

Activity was assessed immediately following oxytocin or vehicle treatment in a 2-h test in a photocell-equipped automated open field (41 cm × 41 cm × 30 cm; Versamax System, AccuScan Instruments, Columbus, OH). Parameters included total distance and time spent in the center region of the chamber. Activity chambers were contained inside sound-attenuating boxes, equipped with ceiling lights and fans.

2.7. Statistical analysis

Data were analyzed using StatView software (SAS, Cary, NC). For the BALB/cByJ model, groups were compared with repeated measures analysis of variance (ANOVA), with factors treatment, cohort group (for the sub-chronic regimen), and side of social test box. For the C58/J model, data from the social task were analyzed with repeated measures ANOVAs, with factors treatment, sex, time of test, and side of social test box. Separate repeated measures ANOVAs were conducted for each sex to determine oxytocin effects on sociability in male and female mice. Within-treatment repeated measures ANOVAs were used to determine social preference. Activity data were analyzed with repeated measures ANOVAs, with factors treatment, sex, and time (5-min intervals across a 2-h test). For all comparisons, significance was set at $p < 0.05$.

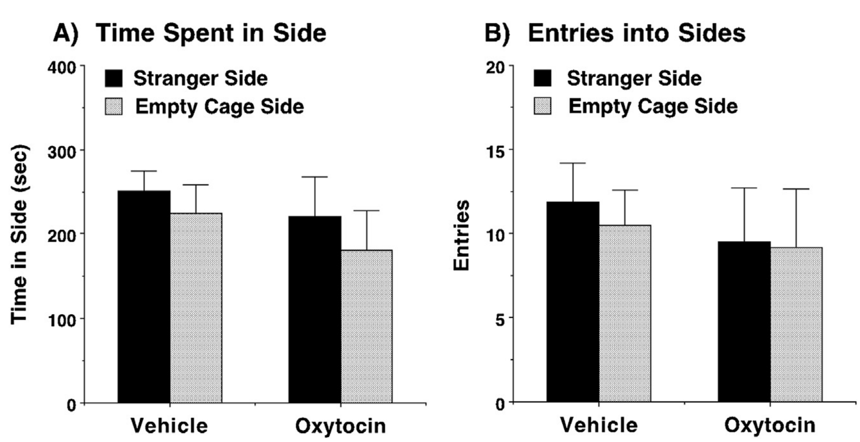

Fig. 2. Lack of prosocial effects of oxytocin with acute treatment in male BALB/cByJ mice. Oxytocin (1.0 mg/kg) was administered 50 min before evaluating social approach in the three-chambered choice test. Data shown are means (+SEM) for 6 mice per treatment group.

3. Results

3.1. Oxytocin effects in the BALB/cByJ model

We previously reported low sociability in adolescent male BALB/cByJ mice (Moy et al., 2007). Considering the established connection between oxytocin and social behavior, we predicted that oxytocin would rescue this deficit in sociability. Surprisingly, we found that acute treatment with oxytocin failed to induce significant social preference (Fig. 2A) or increase exploration (Fig. 2B) in BALB/cByJ Cohort 1. Because acute oxytocin treatment lacked prosocial efficacy, and other behavioral studies have shown beneficial effects of repeated oxytocin administration (Bowen et al., 2011; Cushing and Carter, 2000), we next tested a sub-chronic oxytocin regimen. Following a sub-chronic regimen, two cohorts of mice (BALB/cByJ Cohorts 2 and 3) treated with oxytocin demonstrated a significant preference for social proximity in a three-chambered choice task, conducted 24 h following the final dose of oxytocin (Fig. 3). A repeated measures ANOVA on time spent in each side chamber by the two cohort groups revealed a highly significant main effect of side [$F(1,53) = 7.72$, $p = 0.0075$], and a treatment × side interaction that approached significance [$F(1,53) = 3.34$, $p = 0.0731$] (Fig. 3A and B). Oxytocin also led to a significant preference for sniffing directed toward the cage containing the stranger mouse, versus the empty wire cage, in both cohort groups [within-treatment comparisons following significant main effect of side, $F(1,51) = 8.61$, $p = 0.005$, and treatment × side interaction, $F(1,51) = 4.59$, $p = 0.037$] (Fig. 3C and D). Mice given sub-chronic exposure to oxytocin showed a small increase in number of entries during the test [treatment × side interaction, $F(1,53) = 4.81$, $p = 0.0327$],

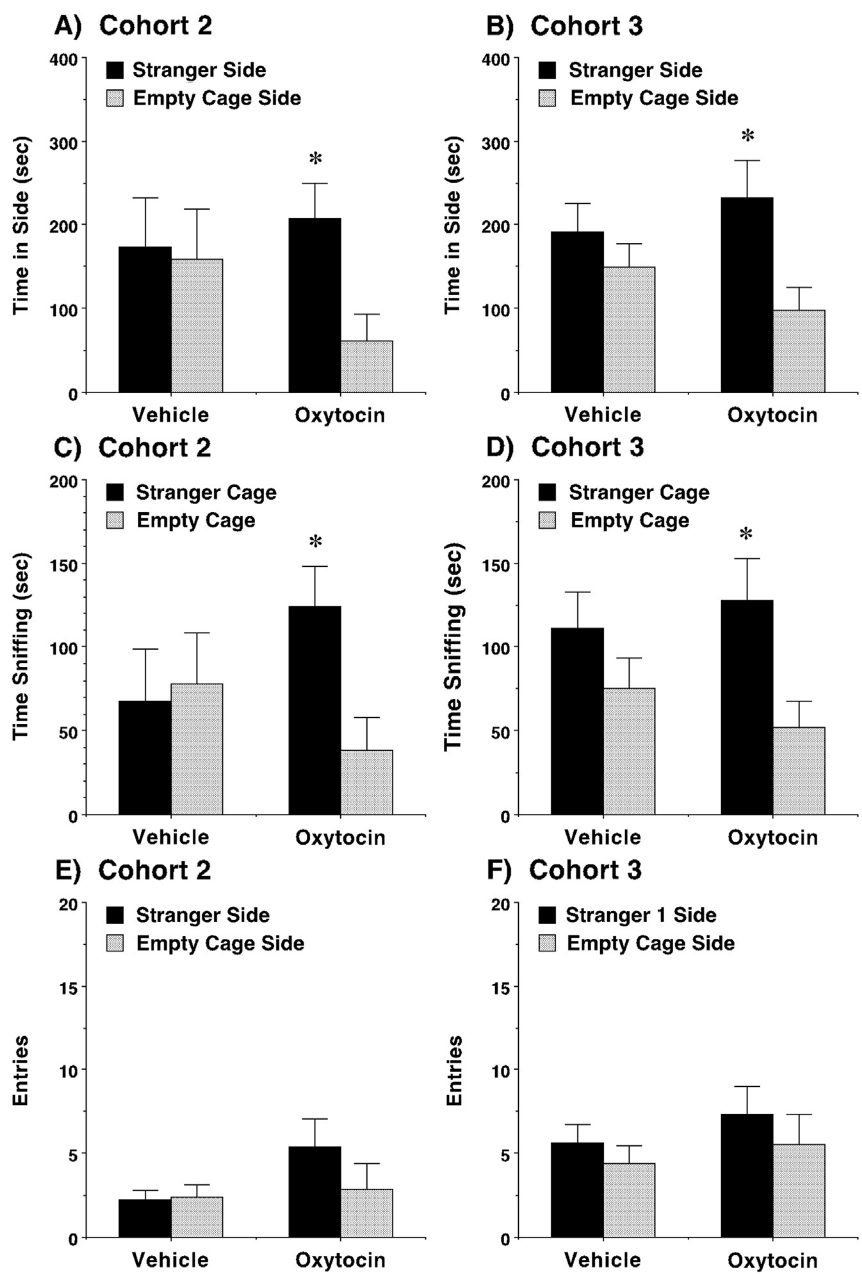

Fig. 3. Prosocial effects of oxytocin with sub-chronic treatment in male BALB/cByJ mice. The sub-chronic regimen consisted of four doses of either vehicle or oxytocin (1.0 mg/kg) across an 8–9 day period, with at least 48 h between each IP injection. Subjects were tested for sociability in the three-chambered choice test 24 h following the final dose. $N = 12$–18 per group. Sniffing data are missing from two mice in Cohort 2 due to experimenter error. *$p < 0.05$, within-group comparison.

although post-hoc comparisons were not significant (Fig. 3E and F). No significant effects of cohort were found for any measure.

3.2. Prosocial oxytocin effects in the C58/J model

Previous work in our laboratory has shown that both male and female C58/J mice exhibit social deficits, as well as overt repetitive behavior (Moy et al., 2008; Ryan et al., 2010). Because the acute oxytocin treatment did not have significant prosocial effects in the BALB/cByJ model, we only tested C58/J mice with the sub-chronic regimen. In contrast to our findings in BALB/cByJ, sub-chronic treatment with oxytocin in C58/J did not reverse lack of social preference 24 h following the final drug dose (Fig. 4). However, one study has shown that prosocial effects of repeated oxytocin administration can be observed up to 13 days post-treatment (Bowen et al., 2011). Thus, C58/J mice were given a second social approach test, with half of the mice retested 1 week after sub-chronic oxytocin treatment (C58/J Cohort 1), and half of the mice retested 2 weeks after treatment (C58/J Cohort 2). Strikingly, significant prosocial oxytocin effects emerged in these follow-up tests [within-treatment comparisons following significant main effect of side, $F(1,56) = 11.96$, $p = 0.001$; treatment × side interaction, $F(1,56) = 7.19$, $p = 0.0096$; and main effect of sex, $F(1,56) = 4.03$, $p = 0.0497$; no significant effect of week]. Notably, there were no effects of retest (week of testing) on side preference in the vehicle treatment groups.

Separate repeated measures ANOVAs were conducted for the male and female groups to determine whether the prosocial efficacy of oxytocin was dependent upon sex. In the male mice, significant sociability was observed in the oxytocin-treated group retested at the 2-week, but not the 1-week, time point [main effect of side, $F(1,28) = 4.98$, $p = 0.0339$; and main effect of week, $F(1,28) = 6.59$, $p = 0.0159$] (Fig. 4A). In contrast, strong preference for spending time in the side with the stranger mouse was observed in the oxytocin-treated female groups at both time points [main effect of side, $F(1,28) = 7.05$, $p = 0.0129$; and treatment × side interaction, $F(1,28) = 8.21$, $p = 0.0078$; no significant effect of week] (Fig. 4B).

We have previously found that the measure of time spent sniffing the wire cages is not as robust an index for social deficits as

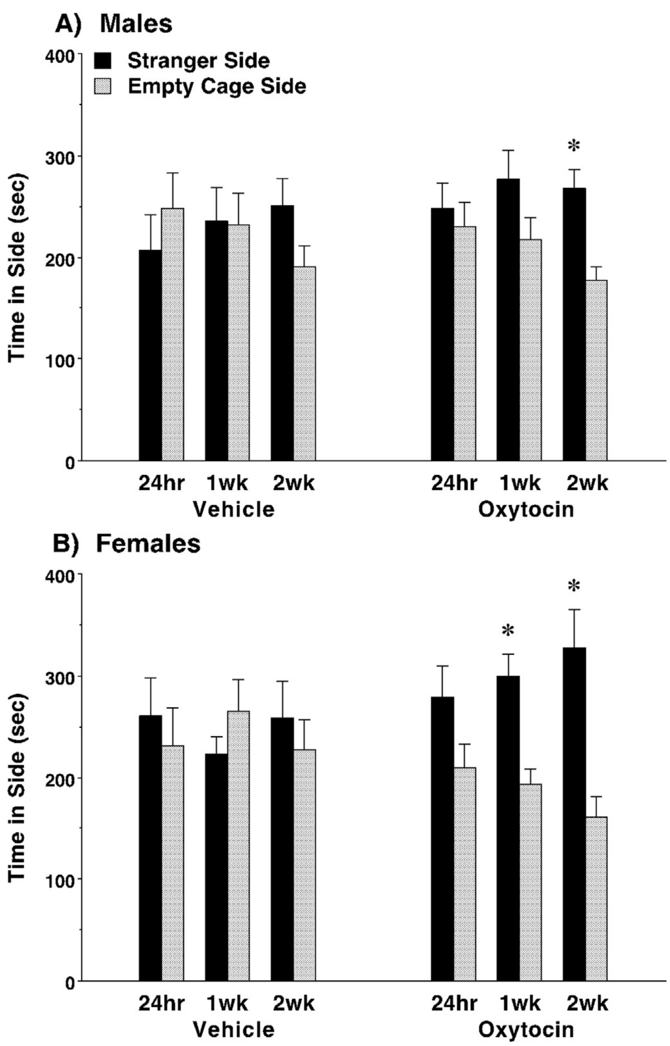

Fig. 4. Prosocial effects of sub-chronic oxytocin in C58/J mice. The sub-chronic regimen consisted of four doses of either vehicle or oxytocin (1.0 mg/kg) across an 8–9 day period, with at least 48 h between each IP injection. Subjects were tested for sociability 24 h following the final dose ($N = 16$ per sex, per treatment). Half of the mice were further tested 1 week (1 wk) after the sub-chronic oxytocin regimen, and the remaining mice were tested 2 weeks (2 wk) following treatment. Data were lost for one female subject at the 24-h time point due to equipment failure. *$p < 0.05$, within-treatment group comparison.

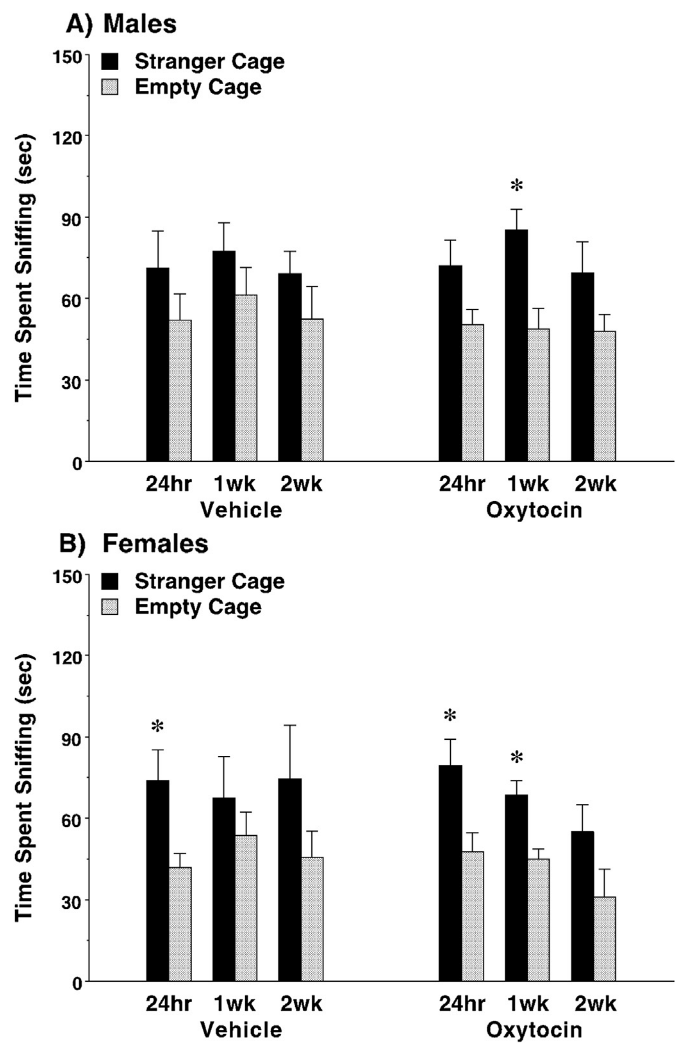

Fig. 5. Effects of sub-chronic oxytocin on time spent sniffing the stranger cage or empty cage. The sub-chronic regimen consisted of four doses of either vehicle or oxytocin (1.0 mg/kg) across an 8–9 day period, with at least 48 h between each IP injection. Subjects were tested for sociability 24 h following the final dose ($N = 16$ per sex, per treatment). Half of the mice were further tested 1 week (1 wk) after the sub-chronic oxytocin regimen, and the remaining mice were tested 2 weeks (2 wk) following treatment. *$p < 0.05$, within-treatment group comparison.

time spent in each side chamber (Moy et al., 2008; Ryan et al., 2010). In the present study, repeated measures ANOVAs did not identify any significant treatment effects on sniffing in either the male or female groups (Fig. 5). However, it is notable that none of the male mice treated with vehicle demonstrated significant preference for sniffing at the stranger cage, while the oxytocin-treated male mice had overt sniffing preference for the social stimulus, albeit only at the 1-week time point [post-hoc tests following main effect of side, $F(1,28) = 13.65$, $p = 0.0009$] (Fig. 5A). In contrast, both the vehicle and oxytocin groups of female mice showed significant social preference 24 h after the last dose of the drug regimen [main effect of side, $F(1,30) = 14.46$, $p = 0.0007$] (Fig. 5B). At the 1-week time point, only the oxytocin-treated female group still demonstrated sniffing preference for the stranger cage [main effect of side, $F(1,28) = 11.9$, $p = 0.0018$].

Lastly, there was a significant effect of retest on the numbers of entries, indicating that the amount of exploration in the social approach test changed with acclimation to the task [main effect of test, $F(1,55) = 106.57$, $p < 0.0001$]. However, the sub-chronic oxytocin regimen did not have any effects on this measure (Fig. 6), suggesting that the increased sociability was not due to a general increase in activity during the test.

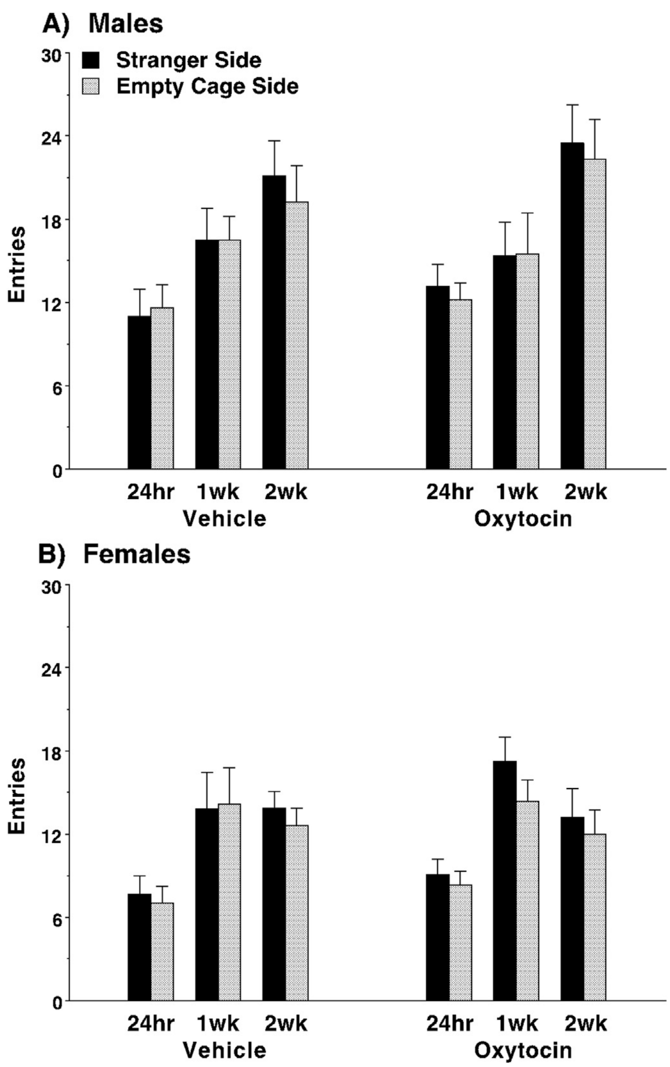

Fig. 6. No effects of sub-chronic oxytocin on number of entries in C58/J mice. The sub-chronic regimen consisted of four doses of either vehicle or oxytocin (1.0 mg/kg) across an 8–9 day period, with at least 48 h between each IP injection. Subjects were tested for sociability 24 h following the final dose ($N = 16$ per sex, per treatment). Half of the mice were further tested 1 week (1 wk) after the end of the oxytocin regimen, and the remaining mice were tested 2 weeks (2 wk) following the final dose. Data were lost for one female subject at the 24-h time point due to equipment failure.

3.3. Oxytocin and repetitive behavior in the C58/J model

In addition to social and communication deficits, the C58/J strain provides a model of abnormal repetitive behavior, relevant to the core diagnostic indicators for autism (Moy et al., 2008; Ryan et al., 2010). In the present study, the effects of both acute (C58/J Cohort 3) and sub-chronic (C58/J Cohort 1) oxytocin treatment were evaluated against motor stereotypy (backflipping and repeated jumping), a lower-order form of repetitive behavior (Fig. 7). Because no significant effects of sex were determined for stereotypy following either dosing regimen, data for male and female mice were combined. As shown in Fig. 7A, an acute single-dose of oxytocin (1.0 mg/kg) decreased levels of repetitive behavior, with significant effects emerging in the last 10 min of the recording session [post-hoc comparisons following main effect of treatment, $F(1,15) = 11.26$, $p = 0.0043$; and time, $F(1,15) = 11.54$, $p = 0.004$]. This dose also decreased locomotion across the first and second halves of the recording session [main effect of treatment, $F(1,15) = 19.41$, $p = 0.0005$; and time, $F(1,15) = 9.35$, $p = 0.008$] (Fig. 7B). However, levels of grooming were increased by oxytocin [main effect of treatment, $F(1,15) = 9.01$, $p = 0.009$], indicating that the neuropeptide did not induce general decreases in all behaviors (Fig. 7C). Sub-chronic treatment with oxytocin did not have any persistent effects on motor stereotypy, locomotion, or grooming in the C58/J mice (Fig. 7D–F).

Although the results from the acute regimen showed that oxytocin could significantly reduce aberrant repetitive behavior, the concomitant decrease in locomotion indicated that acute oxytocin might also have sedative-like effects. Therefore, the effects of oxytocin on general activity levels were examined in C58/J Cohort 4 (Fig. 8). Because repeated measures ANOVAs did not reveal significant effects of sex on measures from the activity test, data from male and female mice were combined. As shown in Fig. 8A, there were no significant effects of oxytocin on locomotion in an open field test across a 2-h session. Furthermore, oxytocin did not increase time spent in the center regions, suggesting the neuropeptide did not have anxiolytic-like effects in C58/J (Fig. 8B).

4. Discussion

This study provided the first evidence that peripherally administered oxytocin could reverse the social deficits exhibited by two mouse models with face validity to core ASD symptoms (i.e. BALB/cByJ and C58/J mice). We also observed that exogenous oxytocin could attenuate motor stereotypy in C58/J mice, at a dose that did not induce statistically significant hypoactivity in an open field test. Importantly, the ability of oxytocin to influence these ASD-like behaviors was critically dependent on the dosage regimen (i.e. sub-chronic but not acute oxytocin could improve social deficits, and vice versa for repetitive behavior in C58/J). Our preclinical findings complement clinical studies that demonstrate the beneficial effects of oxytocin in ASD subjects, and demonstrate the importance of treatment regimen, genotype, and sex in modulating oxytocin efficacy.

To date, most clinical studies have only tested acute single-dose oxytocin treatment in ASDs. For example, Hollander and colleagues found that oxytocin infusion improved social cognition and reduced repetitive behavior in ASD subjects (Hollander et al., 2007, 2003). Two other studies determined that acute intranasal oxytocin improved emotion recognition in young ASD subjects (ages 12–19), and led to increased eye gaze and more appropriate social behavior

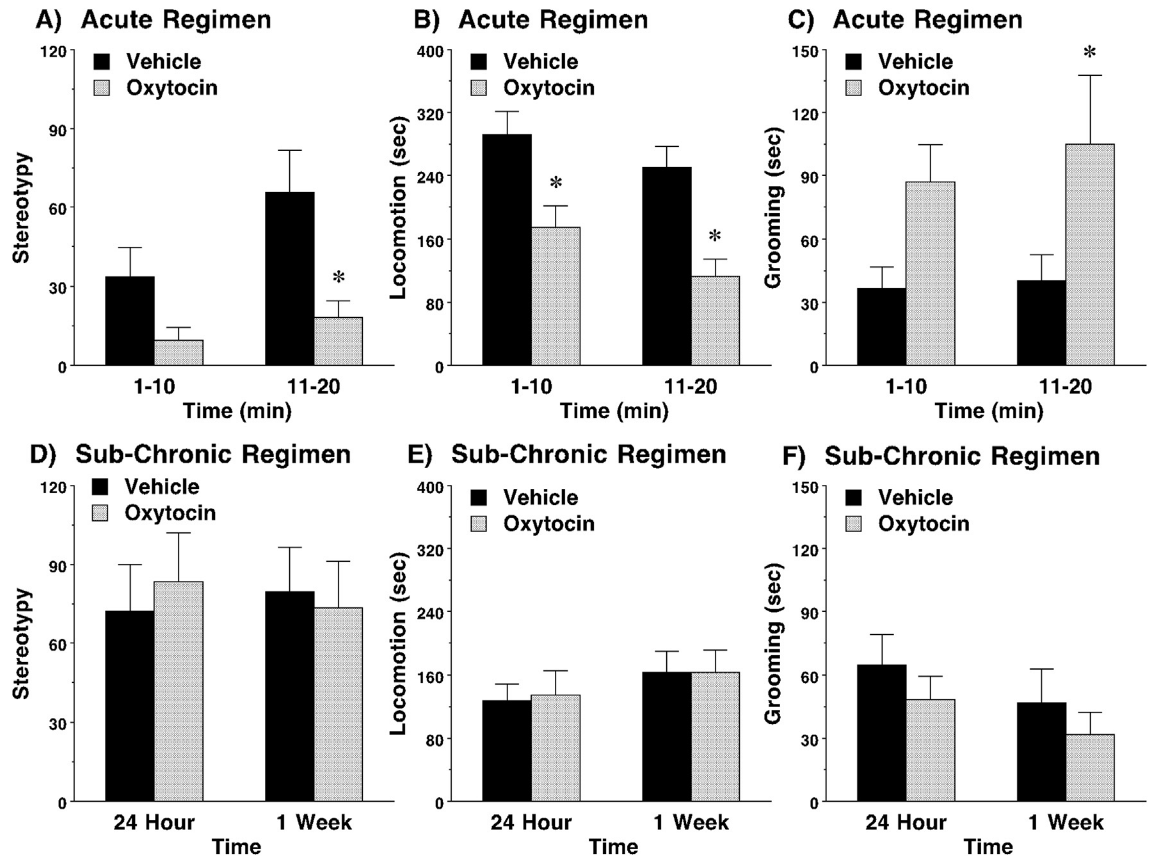

Fig. 7. Effects of acute and sub-chronic oxytocin on motor stereotypy in C58/J mice. A–C) Oxytocin (1 mg/kg) or vehicle was administered 50 min before a 20-min session. Means (+SEM) are presented for the first and second 10-min intervals. Data for five mice with zero levels of stereotypy after vehicle treatment were not included. Remaining number of mice were $N = 16$ (9 males and 7 females). D–F) The sub-chronic regimen consisted of four doses of either vehicle or oxytocin (1.0 mg/kg) across an 8–9 day period, with at least 48 h between each IP injection. Subjects were first tested for sociability 24 h or 1 week following the final dose, and then given a 30-min session for repetitive behavior following each test. Data are presented for the final 10 min. $^{*}p < 0.05$.

in high-functioning ASD subjects (Andari et al., 2010; Guastella et al., 2010). More recently, a 6-week randomized controlled trial of twice daily intranasal oxytocin in adults with ASD demonstrated efficacy on social perception and quality of life, and a trend for improvement in lower-order repetitive behavior (Anagnostou et al., 2012). Although this longer-term study showed that daily oxytocin is well tolerated and has therapeutic potential, the treatment did not have efficacy in the primary outcome measures of social cognition/function or higher-order repetitive behavior. Interestingly, clinical studies in schizophrenia (another disorder characterized by social deficits) found improvement in symptoms, including social cognition, after 2–3 weeks of daily intranasal oxytocin administration (Feifel et al., 2010; Pedersen et al., 2011). Overall, these clinical studies support oxytocin as a promising intervention, but further work is necessary to determine optimal treatment regimens and limiting factors for therapeutic efficacy. Considering the genetic heterogeneity in the human population and the differential prosocial efficacy of oxytocin treatment in BALB/cByJ and C58/J, genetic background is likely to be an important factor in modulating oxytocin response in individuals with ASD.

In the C58/J model, the sub-chronic oxytocin regimen induced prosocial effects that emerged 1–2 weeks following treatment, dependent on sex. In particular, significant sociability emerged 2 weeks post-treatment in male C58/J mice, but was observed at the 1-week and 2-week post-treatment time points in female C58/J mice. These relatively long-term effects of sub-chronic oxytocin on social behavior are consistent with findings from Bowen and colleagues showing that 10 days of once-daily oxytocin (1 mg/kg, IP) modestly improved sociability in rats after a 13-day washout period (Bowen et al., 2011). Although the neurobiology underlying the prosocial effects of sub-chronic oxytocin treatment remains to be elucidated, one contributing mechanism may be the up-regulation of the endogenous oxytocin system within the hypothalamus (e.g. increased oxytocin receptor gene expression and oxytocin secretion) with repeated exposures (Bowen et al., 2011). Another study has suggested that strong, chronic stimulation of the oxytocin system promotes the reversible structural plasticity/remodeling of oxytocinergic neurons and their associated glial cells in adult hypothalamus, leading to altered function and increased oxytocin secretion (Theodosis, 2002). Moreover, ovarian hormones stimulate hypothalamic oxytocin and oxytocin receptor expression, and enhance the electrical activity of oxytocin neurons (Armstrong et al., 2002; Bale et al., 1995; Coirini et al., 1992; de Kloet et al., 1986; Israel and Poulain, 2000; Miller et al., 1989; Patisaul et al., 2003), which may account for the higher efficacy of exogenous oxytocin in female versus male C58/J mice. The sexually dimorphic effects might also be related to sex-specific dependence on either the oxytocinergic or vasopressinergic systems, as seen in prairie voles (Cushing and Carter, 2000; Insel and Hulihan, 1995; Yamamoto et al., 2004). Taken together, these observations suggest that repeated oxytocin administration primes the oxytocin system to become more sensitive to endogenous oxytocin signaling. Because deficiencies in the oxytocin pathway have been associated with ASDs, sensitization of the oxytocin system may improve sociability in autistic people.

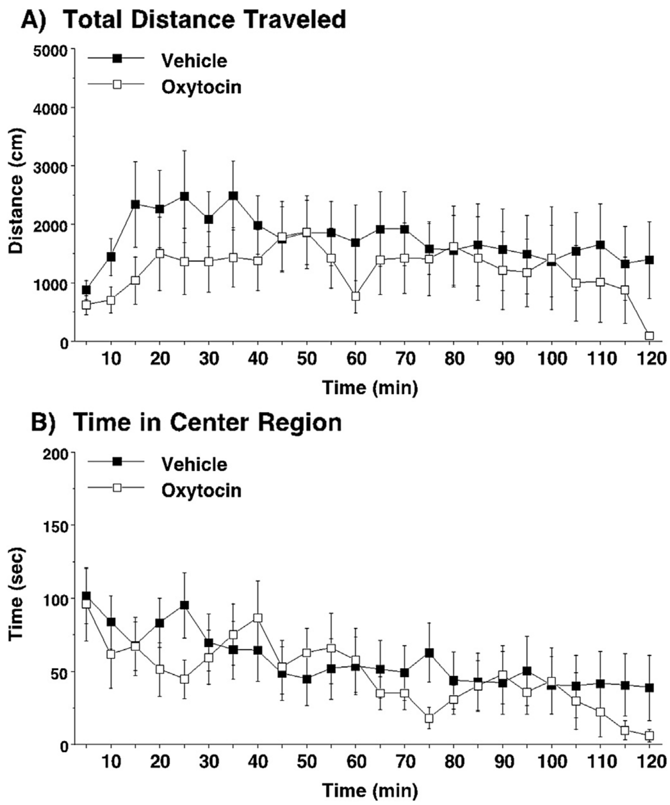

Fig. 8. Lack of oxytocin effects on activity and anxiety-like behavior in an open field test. Oxytocin (1.0 mg/kg) or vehicle was administered immediately before the start of a 2-h open field test. Data are means (+SEM) for 16 C58/J mice (8 males and 8 females), each tested once with vehicle and once with oxytocin, using a crossover design.

Considering the lack of drug therapies to ameliorate the core symptoms of ASDs, the oxytocin receptor is an attractive target, particularly for addressing the profound social deficits observed in autism. Yet, how the oxytocin system regulates social behavior is poorly understood, perhaps because this system can influence and be influenced by other neurochemical systems. For example, serotonin receptors and glutamate receptors regulate oxytocin release (Busnardo et al., 2012; Jørgensen et al., 2003; Pampillo et al., 2001; Saydoff et al., 1991). It is notable that the few drugs that exhibit prosocial efficacy in mouse models of social deficits directly or indirectly target the serotonergic system (e.g. MDMA, buspirone, and fluoxetine) (Chadman, 2011; Gould et al., 2011; Thompson et al., 2007) and the glutamatergic system (e.g. D-serine, MP-10, papaverine, GRN-529, and AMPAKINEs) (Grauer et al., 2009; Jacome et al., 2011; Labrie et al., 2008; Silverman et al., 2013, 2012b). Thus, one could speculate that an underlying mechanism for the prosocial effects of these agents is the modulation of oxytocin secretion. Conversely, oxytocin itself has been shown to influence several neurochemical systems, including the serotonergic, glutamatergic, dopaminergic, and GABAergic systems in multiple brain regions (e.g. hypothalamus, amygdala, and hippocampus) (Brussaard et al., 1996; Eaton et al., 2012; Melis et al., 2009; Ninan, 2011; Rosenfeld et al., 2011; Sala et al., 2011; Succu et al., 2011; Theodosis et al., 2006; Yoshida et al., 2009). Therefore, oxytocin treatment may stimulate a feed-forward loop through the modulation of these neurochemical systems that provide feedback input to the oxytocinergic system, which could contribute to prosocial efficacy.

In contrast to the findings on social approach, we did not observe any sexually dimorphic effects of oxytocin administration on repetitive behavior in the C58/J model. For both male and female C58/J mice, abnormal repetitive behavior was decreased by acute, but not sub-chronic, treatment with oxytocin. It is possible that the dose of oxytocin utilized in the sub-chronic regimen was not optimal for persistent effects on repetitive behavior. Other studies examining oxytocin effects in animal models have reported bell-shaped dose response curves, with higher doses of oxytocin exhibiting less efficacy than more moderate doses (Arletti and Bertolini, 1987; Kovács et al., 1985). Further, the repetitive behavior analysis after sub-chronic oxytocin treatment was conducted at the 24-h and 1-week time points, while the strongest prosocial oxytocin effects were observed at the 2-week time point. Evaluation of repetitive behavior at later time points could reveal the emergence of oxytocin treatment effects. Although our finding of decreased motor stereotypy following acute oxytocin treatment is in line with one clinical study in adults with autism or Asperger's syndrome (Hollander et al., 2003), an important caveat is possible effects on activity, especially sedative-like action (Uvnäs-Moberg et al., 1994). In the present study, acute oxytocin led to a significant decrease in locomotion and an increase in grooming during the test for repetitive behavior, which could have confounded drug effects on motor stereotypy. While the results from the open field test imply that the acute effects of oxytocin on motor stereotypy were not simply due to hypoactivity or anxiolytic-like effects, we observed substantial variability in these measures and a possible trend for reduced locomotion during multiple time intervals. Further research will be necessary to clarify the effects of oxytocin treatment on repetitive behavior in the C58/J model, including an evaluation of dose–response.

In conclusion, sub-chronic oxytocin treatment reverses sociability deficits in the BALB/cByJ and C58/J mouse models of ASD-relevant phenotypes. These inbred mouse strains provide an attractive translational platform for screening drugs targeting the oxytocin signaling pathway and for investigating mechanisms underpinning the efficacy of oxytocin against core ASD symptoms. Our ongoing studies include testing the dose-dependence and longer-term persistence of oxytocin effects on social and repetitive behavior. Using these models, future studies analyzing the molecular and neurobiological changes in response to oxytocin treatment could provide important insights into social behavior and new avenues for drug discovery in ASDs and other disorders characterized by impaired social function.

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